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Minireview

Organelle Structure, Function, and Inheritance in Yeast: A Role for Fatty Acid Synthesis?

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Although fatty acids are generally acknowledged to be essential, impaired fatty acid biosynthesis is not readily associated with morphological defects. This view has been changed by two recent studies of conditional yeast mutants that affect key steps of fatty acid synthesis. Characteristically, these mutants were isolated in screens unrelated to lipid metabolism (Table 1). This minireview focuses on the link between fatty acid synthesis and organelle structure, function, and inheritance (Warren and Wickner, 1996). Whether this link is a direct one or, instead, the observed phenotypes are indirect manifestations of a cell that fails to properly synthesize and assemble its membrane components, however, is not yet resolved. We describe the newly discovered mutant phenotypes in the context of earlier observations that fatty acid desaturation is required for mitochondrial movement and inheritance and that the addition of activated fatty acids to a reconstituted secretory transport assay promotes vesicle budding and fusion. In an attempt to correlate the different mutant phenotypes, we discuss a model that implies different classes of fatty acids in stabilizing or resolving highly curved membrane structures, such as those formed as intermediates during membrane budding and fusion.

Figure 1 outlines the general pathway of fatty acid uptake, biosynthesis, and degradation established for the yeast Saccharomyces cerevisiae and, to a lesser extent, for Schizosaccharomyces pombe. In this scheme, the synthesis of saturated long-chain fatty acids (LCFAs; C_{14}–C_{18}) is central to their subsequent desaturation, to their elongation to very long-chain fatty acids (VLCFA; $>C_{22}$), to peroxisomal degradation through β-oxidation, and to their function in protein acylation. De novo synthesis of LCFAs is catalyzed by the concerted action of four different enzyme systems: acetyl-CoA carboxylase (Acc1p/Mtr7p and Cut6p), fatty acid synthetase (Fas1p, Fas2p subunits, and Lsd1p), elongase (Elo1p), and fatty acid desaturase (Ole1p/Mdm2p). The rate-limiting step of LCFA synthesis is under the control of the first of these, acetyl-CoA carboxylase. This biotin-containing enzyme catalyzes the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA, which then serves as the two-carbon-unit donor for acyl chain elongation by fatty acid synthetase and elongase. The end product of this reaction is palmitoyl- and stearoyl-CoA. Uptake and activation of exogenous fatty acids by S. cerevisiae requires at least four acyl-CoA synthetases (Faa1p–Faa4p). Transport of LCFAs across the peroxisomal membrane depends on two ATP-binding cassette transporters, Pat1p and Pat2p, while peroxisomal β-oxidation of medium chain fatty acids (MCFAs; C_8–C_{12}) requires their activation inside peroxisomes by an acyl-CoA synthetase, Faa2p. Myristoylation of membrane-associated proteins is catalyzed by Nmt1p (for review on lipid synthesis in yeast, see Kohlwein et al., 1996).

S. cerevisiae Fatty Acid Synthesis Mutants

Mutants defective in fatty acid synthesis were isolated in the early 1970s in screens for cells that can grow only when fatty acids are added to the growth medium. All the mutants selected by this approach, *ole1*, *fas1*, *fas2*, *acc1*, and *acc2* (defective in apoenzyme-biotin ligase) were completely rescued by appropriate fatty acid supplementation. Subsequent cloning and disruption of acetyl-CoA carboxylase, however, revealed that cells lacking Acc1p are not viable even if fatty acids are supplemented (Hasslacher et al., 1993). This observation suggested that acetyl-CoA carboxylase performs a second essential function in addition to the synthesis of LCFAs.

Confirming this conclusion, Tartakoff and collaborators isolated a temperature-sensitive allele of acetyl-CoA carboxylase (*mtr7*) that is not rescued by LCFA supplementation (Schneiter et al., 1996). *mtr7* cells accumulate nuclear poly(A)$^+$ RNA (*m*RNA *tr*ansport mutants), suggesting that the nonsupplementable function of acetyl-CoA carboxylase is directly or indirectly related to the structure and function of the nuclear envelope/nuclear pore complex. Unlike the fatty acid auxotrophic acetyl-CoA carboxylase mutant cells, *mtr7* cells display a striking separation of inner and outer nuclear membranes that is accompanied by the formation of vesicle-like structures within the newly formed intermembrane space (Figure 2). An in vitro–generated biotin-deficient point mutant allele of *ACC1* does not rescue the lethality of a null allele, suggesting that the essential function of acetyl-CoA carboxylase depends on its enzymatic activity. Comparison of the fatty acid chain length profile of *acc1* and *mtr7* strains revealed that *mtr7*, but not *acc1*, affects the synthesis of VLCFAs (C_{26}), suggesting that malonyl-CoA-dependent elongation of LCFAs to

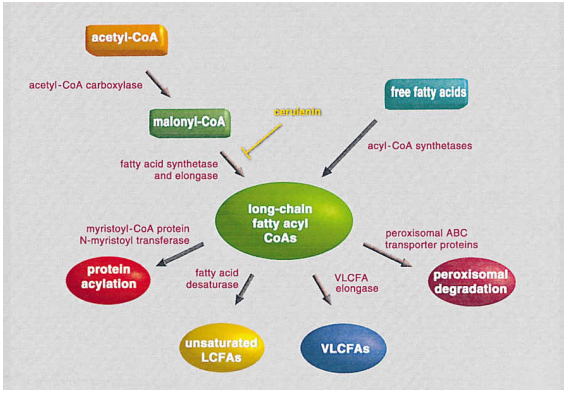

Figure 1. Pathways of Fatty Acid Synthesis and Degradation in S. cerevisiae

See text for details.

Table 1. Yeast Fatty Acid Synthesis Mutants

Enzyme	Organism	Mutant	Morphological Phenotype	Rescue	Reference
acetyl-CoA carboxylase	S. cerevisiae	*acc1* *a*cetyl-*C*oA *c*arboxylase	none described	fatty acids	1
		mtr7 *m*RNA *t*ransport	separation of inner and outer nuclear membranes	—	2
	S. pombe	*cut6* cell *u*ntimely *t*orn	impaired separation of nonchromsomal nuclear components	—	3
fatty acid synthetase	S. cerevisiae	*fas1*, *fas2* *f*atty *a*cid *s*ynthetase	none described	fatty acids	4
	S. pombe	*lsd1* *l*arge and *s*mall *d*aughter nuclei	impaired separation of nonchromosomal nuclear components	fatty acids	3
fatty acid desaturase	S. cerevisiae	*mdm2* *m*itochondrial *d*istribution and *m*orphology	defect in segregating mitochondria into the daughter cell	unsaturated fatty acids	5

References: 1. Roggenkamp et al., 1980; 2. Schneiter et al., 1996; 3. Saitoh et al., 1996; 4. Schüller et al., 1992; 5. Stewart and Yaffe, 1991.

VLCFAs is the nonsupplementable function of mutants that lack acetyl-CoA carboxylase activity. Attempts to complement the mutant phenotype of *mtr7* by supplementation with a mixture of LCFAs and VLCFAs were unsuccessful, possibly due to the poor uptake and/or activation of the latter extremely hydrophobic compounds. Thus, the originally isolated fatty acid auxotrophic *acc1* alleles are likely to be leaky and to synthesize sufficient malonyl-CoA to sustain VLCFA synthesis.

VLCFAs, of which $C_{26:0}$ is the most abundant in yeast, comprise approximately 1% of the total fatty acid content. Their synthesis is essential since they form a structurally important part of the ceramide moiety of sphingolipids and the lipid domain of glycosylphosphatidylinositol (GPI)-anchored proteins. The structural importance of the very long acyl chain length in sphingolipid function is underlined by the observation that strains which survive without synthesizing ceramide produce novel C_{26} fatty acid–substituted inositol glycerophospholipids that structurally mimic sphingolipids (Lester et al., 1993).

mtr7 mutants display the characteristic nuclear envelope phenotype; however, fatty acid auxotrophic *acc1* mutants and wild-type cells treated with cerulenin, an inhibitor of fatty acid synthetase, do not. This observation suggests that the synthesis of C_{26} is directly or indirectly required to stabilize the 180° turn of the nuclear membrane at the nuclear pore complex. A possible structural function of VLCFA-substituted lipids in stabilizing such highly curved membrane structures is suggested by in vitro studies of their phase properties. To determine whether such lipids are also found at places of high membrane curvature in vivo, a thorough characterization of their structure and distribution is in order.

A role of fatty acids in organelle inheritance is suggested by the observation that *mdm2*, a mutant defective in *m*itochondrial *d*istribution and *m*orphology, is allelic to *OLE1*, the Δ9 fatty acid desaturase structural gene (Stewart and Yaffe, 1991). *mdm2* cells are temperature-sensitive specifically for the transfer of mitochondria into the growing bud. Nuclei and vacuoles are faithfully transmitted to developing buds; secretion, nuclear division, and cytokinesis also proceed normally. When *mdm2* cells are shifted to the nonpermissive temperature, the levels of unsaturated fatty acids ($C_{16:1}$ and $C_{18:1}$) decline ~2.5-fold and the levels of precursors $C_{16:0}$ and $C_{18:0}$ fatty acids increase. Concomitantly, the reticular mitochondrial network fragments and mitochondrial vesicles aggregate in the mother cell. Interestingly, the mutation is cell cycle–specific with an execution point in late G1 or early S phase. While the molecular details and control of this mitochondrial movement have yet to be described, the fact that oleic acid complements the temperature-sensitive growth and mitochondrial distribution defects of *mdm2* suggests that unsaturated fatty acids are essential for an as yet undefined probably

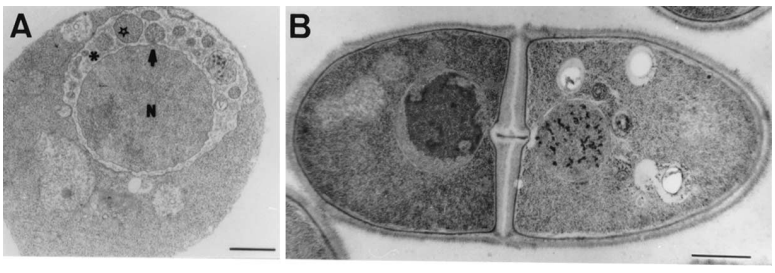

Figure 2. Morphological Phenotypes of Yeast Fatty Acid Synthesis Mutants

Separation of inner and outer nuclear membranes (asterisks) and formation of vesicle-like structures (open star) in the intermembrane space of S. cerevisiae acetyl-CoA carboxylase mutants are shown in (A). The arrow indicates a nuclear pore complex (reproduced by copyright permission from Schneiter et al., 1996). The nuclear segregation phenotype displayed by S. pombe fatty acid synthetase mutants is shown in (B). The two nuclei are connected to each other through the thin fiber-like structure that runs through the septum (reproduced from the Journal of Cell Biology, 1996, *134*, p. 953; by copyright permission of The Rockefeller University Press). Bar in (A), 0.6 μm; (B), 1 μm.

membrane-associated process. Since cardiolipin, a lipid predominantly found in the inner mitochondrial membrane, is particularly rich in unsaturated fatty acids, it will be interesting to know whether mutants that specifically affect cardiolipin synthesis also display an mdm phenotype (Zinser et al., 1991).

S. pombe Mutants

Fission yeast conditional mutants that are defective in the proper coordination of nuclear division and cytokinesis have been isolated by examining their cytological phenotypes. Mutants in which septation and subsequent cytokinesis takes place—although the preceding nuclear division is absent or abnormal—have been termed *cut* (*c*ell *u*ntimely *t*orn). Yanagida and colleagues described two novel mutants that display the *cut* phenotype, albeit at a low frequency (Saitoh et al., 1996). More frequently, these mutants, *cut6* and *lsd1*, display a striking nuclear division phenotype that results in daughter nuclei of unequal size (hence designated lsd: large and small daughter). Fluorescence in situ hybridization reveals that sister chromatids are properly separated in lsd cells but appear to be highly compact in one of the two daughter nuclei. Morphological inspection of *lsd1* by thin section electron microscopy reveals an asymmetric nuclear elongation and an unequal separation of nonchromosomal electron-dense nuclear structures. Bisected nuclei are found connected to each other by a fiber-like structure running through the septum (Figure 2). The small nuclei characteristically lack the electron-dense nonchromatin nucleolar domain but contain small dense granules. The size difference of the daughter nuclei thus appears partly to be due to the different degree of chromosome condensation and the impaired separation of nonchromosomal nuclear components.

Both *cut6*$^+$ and *lsd1*$^+$ are essential for viability. *cut6* encodes acetyl-CoA carboxylase and *lsd1* encodes fatty acid synthetase α-subunit; these genes are thus the S. pombe homologs of the budding yeast *ACC1/MTR7* and *FAS2* genes, respectively. Thus, the mutants bear defects in key enzymes of fatty acid synthesis. That the lsd phenotype is indeed due to impaired fatty acid synthesis is supported by the following observations: (a) palmitate supplementation of *lsd1* mutants rescues the lsd phenotype; (b) inhibition of fatty acid synthetase by cerulenin induces the lsd phenotype in wild type cells; and (c) spores containing a disrupted *lsd1* display the lsd phenotype before they cease to divide. Like *mtr7* in S. cerevisiae, S. pombe *cut6* mutants are not rescued by palmitate supplementation. Whether *cut6* displays an mtr phenotype or whether S. cerevisiae *fas2* mutants have an lsd phenotype, however, has not yet been investigated.

A cell cycle dependence of the lsd phenotype is revealed by the fact that a cerulenin-induced block in fatty acid synthesis in S. pombe wild-type cells causes cell death during mitosis but not if cells are arrested in G2. In the presence of cerulenin, viability of G2-arrested *cdc25* mutants remains high but rapidly declines upon release into mitosis.

In all the cases described above, it seems likely that the respective mutant enzyme is inactivated at the nonpermissive temperature, eventually resulting in a decreased intracellular concentration of the corresponding fatty acid. The subsequent shortage of this class of fatty acids might then directly affect the chemical nature of a particular organelle membrane thereby producing the observed phenotype. Alternatively, the phenotype may be an indirect and possibly pleiotropic consequence of a sick cell that fails to assemble important membrane components. For example, a number of membrane-associated proteins require posttranslational acyl chain attachment for activity and/or subcellular targeting. Reduced activity of one of these proteins followed by misregulation of the entire pathway could thus result in the observed morphological phenotypes. That a stringent control over fatty acid synthesis is crucial to cellular function is underlined by the fact that an increased level of fatty acid synthetase is characteristic for a particularly aggressive form of human breast cancer (Kuhajda et al., 1994).

A Specific Role for Fatty Acids in Membrane Budding and Fusion?

The most direct, albeit still elusive, function of fatty acyl-CoA in organelle function is its role in promoting fusion and budding of transport vesicles with Golgi cisternae. In reconstituted transport assays, coated buds that need only be pinched off at their base to release coated vesicles (a process called fission) are observed to accumulate in the absence of acyl-CoA; only when acyl-CoA is added does fission occur, releasing functionally active transport vesicles. In this system, acylation apparently must occur after the coated bud has assembled because the small amount of vesicles formed with coatomer, ARF, and GTP alone could not be increased by preincubation with palmitoyl-CoA (Ostermann et al., 1993). The fact that addition of free fatty acids or a nonhydrolyzable acyl-CoA does not promote fission suggests that some type of acyl chain transfer must occur. Whether this involves protein acylation, localized acyl chain remodeling, or a different as yet unidentified process still awaits clarification.

Membrane Fusion and Mutant Phenotypes: Variations on a Common Theme?

Experimental evidence for a direct relation between mutant phenotypes and impaired fatty acid synthesis is weak. Yet if we propose a direct effect, how could we understand the morphological phenotypes at a mechanistic level? If specific fatty acids are important at specific times and places to ensure proper organelle structure and inheritance, what function could that be? Remarkably, all the morphological defects affect mitochondria and nuclei, organelles that are delineated by double membranes. For *mtr7*, VLCFA synthesis has been proposed to be important to stabilize the turn of the nuclear envelope at the pore complex–membrane interface. This membrane turn ensures the continuity between inner and outer nuclear membranes, which appear disconnected from each other in the mutant. In *mdm2*, the mitochondrial reticulum fragments and aggregates in the mother cell. The fact that the collapsed mitochondria can be stained with a fluorescent dye that is sensitive for the membrane potential indicates that the organization of inner and outer mitochondrial membrane is not grossly altered. On the other hand, in the two fission yeast mutants, *cut6* and *lsd1*, resolution of the dumbbell-shaped nuclear segregation intermediate appears affected. In the in vitro system, finally, acyl-CoA hydrolysis appears to be important for membrane

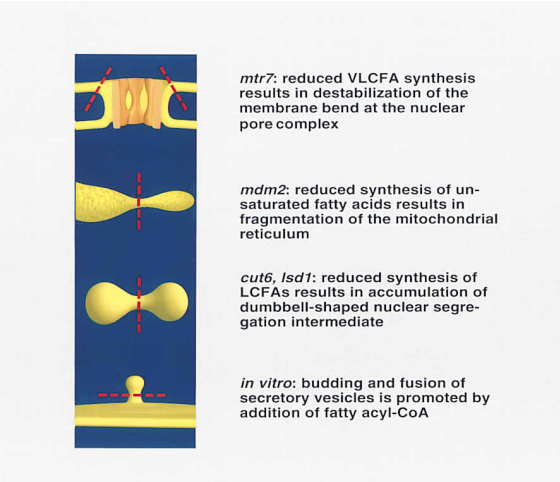

Figure 3. Model to Propose a Possible Requirement of Different Classes of Fatty Acids in Resolving or Stabilizing Highly Curved Membrane Structures

See text for details.

fission and fusion. Thus, at a mechanistic level, it might be speculated that the structural defect common to all these phenomena is linked to either stabilizing or resolving highly curved membrane structures, which are important intermediates in membrane fusion and fission (see Figure 3). That membrane curvature is directly modulated by the lipid composition of a membrane is indicated by biophysical studies. These show that the negative curvature induced by cone-shaped lipids (e.g., cardiolipin) promotes the formation of stalk-like structures that are intermediates in hemifusion. Inverted cones (e.g., lysophospholipids), on the other hand, inhibit fusion when added between the fusing membranes. Thus, different stages of membrane fusion may be controlled (promoted or inhibited) by local alterations of the curvature of one leaflet of the lipid bilayer (see Chernomordik et al., 1997).

In the highly complex intracellular environment, it is likely that important membrane parameters like membrane tension and curvature are simultaneously modulated through both protein assembly on the membrane and remodeling of local membrane lipids. While our understanding of protein assembly on membranes is increasing, the mechanisms that control lipid remodeling and the role of specific lipids in specialized membrane structures are poorly understood. The isolation and phenotypic characterization of these novel yeast mutants in fatty acid metabolism provides an opportunity to study these processes in vivo using genetic and cell biological tools.

Selected Reading

Chernomordik, L.V., Leikina, E., Frolov, V., Bronk, P., Zimmerberg, J. (1997). J. Cell Biol. *136*, 81–93.

Hasslacher, M., Ivessa, A.S., Paltauf, F., and Kohlwein, S.D. (1993). J. Biol. Chem. *268*, 10946–10952.

Kohlwein, S.D., Daum, G., Schneiter, R., and Paltauf, F. (1996). Trends Cell Biol. *6*, 260–266.

Kuhajda, F.P., Jenner, K., Wood, F.D., Hennigar, R.A., Jacobs, L.B., Dick, J.D., and Pasternack, G.R. (1994). Proc. Natl. Acad. Sci. USA *91*, 6379–6383.

Lester, R.L., Wells, G.B., Oxford, G., and Dickson, R.C. (1993). J. Biol. Chem. *268*, 845–856.

Ostermann, J., Orci, L., Tani, K., Amherdt, M., Ravazzola, M., Elazar, Z., and Rothman, J.E. (1993). Cell *75*, 1015–1025.

Roggenkamp, R., Numa, S., and Schweizer, E. (1980). Proc. Natl. Acad. Sci. USA *77*, 1814–1817.

Saitoh, S., Takahashi, K., Nabeshima, K., Yamashita, Y., Nakaseko, Y., Hirata, A., and Yanagida, M. (1996). J. Cell Biol. *134*, 949–961.

Schneiter, R., Hitomi, M., Ivessa, A.S., Fasch, E.-V., Kohlwein, S.D., and Tartakoff, A.M. (1996). Mol. Cell. Biol. *16*, 7161–7172.

Schüller, H.-J., Förtsch, B., Rautenstrauss, B., Wolf, D.H., and Schweizer, E. (1992). Eur. J. Biochem. *203*, 607–614.

Stewart, L.C., and Yaffe, M.P. (1991). J. Cell Biol. *115*, 1249–1257.

Warren, G., and Wickner, W. (1996). Cell *84*, 395–400.

Zinser, E., Sperka-Gottlieb, C.D.M., Fasch, E.-V., Kohlwein, S.D., Paltauf, F., and Daum, G. (1991). J. Bacteriol. *173*, 2026–2034.